

Impact of Beef on Metabolites and Inflammation

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## Study Protocol:

The purpose of this study is to identify metabolic differences in response to consumption of organic grass-fed compared to conventional beef on a wide array of blood borne nutrients including amino acids, lipids, bile acids, and hundreds of other metabolites. This comprehensive analysis is expected to differentiate nutritional and metabolic impacts relevant to human health and provide a foundation for future research. The proposed project will utilize the gold standard research approach of a double-blind, randomized, controlled, cross-over investigation to compare organic and conventional beef. That is, neither investigators nor research participants will know which beef type is being consumed until the study is over and codes identifying the different beef samples are identified, measurements will be made for both beef types in random order for all participants, and variables impacting how participants will respond from one trial to the next will be controlled. A total of 10 healthy adult participants (5 women, 5 men) will be asked to complete a meal test and consume 6-oz grilled (70°C) sirloin steaks (~400 kcal) of either the organic grass-fed or the conventionally grain-fed beef. Selected steaks will come from the same muscle (longissimus lumborum) of the cattle. Blood collection at fasting and hourly for 5 hours after the meal will be obtained. After a 7-day minimum washout period where physical activity and dietary recommendations will be given, participants will repeat the blood draw visit and consume either the organic grass-fed or the conventionally grain-fed beef, whichever was not eaten at the previous blood draw visit. Paired analysis of responses will be conducted to determine differences between the consumption of organic grass-fed beef to conventionally grain-fed beef where participants act as their own controls. This pairing will assist to determine differences in lipid, amino acid and metabolite responses. Kinetics (measures over time) and untargeted and targeted metabolomics (an advance analytical technique to measure 1000's of molecules in a single sample) will be used as a tool to differentiate health impacts of the separate meal tests to investigate the impact of organic grass-fed beef versus conventionally grain-fed beef consumption on fasting and postprandial responses in healthy adults. We expect to identify an array of metabolic differences between beef samples and will identify known impacts of those differences on health to provide a substantive comparison and provide a foundation for future research.

Aim 1: Evaluate postprandial blood markers (glucose, cholesterol, and triglycerides) differences.

Blood markers will be measured via a Picollo Xpress Chemistry Analyzer lipid panel (Abaxis, Union City, CA, USA) at fasting and every hour for a total of four hours after consuming the steaks from each condition. This will provide information about how cattle feeding systems (conventional and grass-fed beef) impact postprandial responses. These acute responses can then be related to broader health impacts, as they are indicative of what long-term consumption could affect.

Aim 2: Evaluate postprandial amino acid differences via targeted metabolomics.

Amino acid concentrations in fasting and postprandial serum samples will be analyzed via a targeted metabolomics approach. This will inform us on the potential differences in amino acid content and amount between conventional and grass-fed beef. Amino acids, both essential and non-essential) play vital roles in numerous metabolic processes and can mediate processes such as glycemic control. Characterizing what amino acids are present and quantifying them will allow for connections to be made on the potential health impacts of consuming each type of beef.

Aim 3: Evaluate postprandial metabolomic differences via untargeted metabolomics.

Metabolites present at fasting and after consumption of the two types of beef will be assessed via an untargeted metabolomics approach. This approach allows us to capture a wider variety of metabolites compared to targeted and aids in the exploratory nature of the purpose and hypothesis. Metabolites found will be identified and connected to metabolic pathways they take part in, as well as the differences in abundance between beef types. This will allow us to potentially discover metabolic pathways being impacted by the consumption of beef not elucidated by the other analysis and identify metabolites that differ between conventional and grass-fed beef.